REPRODUCTION
SPECIAL REVIEW

REPRODUCTIVE TOXICOLOGY

Impact of endocrine disruptors on neurons expressing GnRH or kisspeptin and pituitary gonadotropins

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Abstract

Reproduction is a complex process that is controlled centrally via a network of hypothalamic neurons to modulate the pulsatile release of gonadotropin-releasing hormone (GnRH) and subsequently pituitary gonadotropins. The gonadotropins, luteinizing hormone, and follicle-stimulating hormone, drive gametogenesis and hormone production from the gonads. The hypothalamic-pituitary exchange is controlled by gonadal steroids through negative and positive feedback mechanisms via steroid receptors. Due to the expression of these receptors, GnRH neurons, the hypothalamic neurons that control them, and pituitary gonadotropes are sensitive to exogenous compounds that interact with steroid and nuclear receptors or alter hormone production and metabolism. The compounds, called endocrine-disrupting compounds (EDCs), are ubiquitous and persistent in human environments and could bioaccumulate in the body. EDCs include plasticizers (like bisphenol A), dioxin, polychlorinated biphenyls (PCBs), organochlorine pesticides, flame retardants, and perfluorinated alkyl substances (PFAS). Numerous studies have reported that perinatal, juvenile, or adult exposure to these EDCs, primarily in rats, disrupt the hypothalamic control of pituitary gonadotropin production leading to disruption of gonadal steroid production and estrous cyclicity. The purpose of this review is to evaluate these studies primarily focusing on GnRH and kisspeptin neurons and anterior pituitary gonadotropins and to discuss the need for deeper investigations into the hypothalamic-pituitary-gonadal axis.

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Introduction

Reproduction is governed by the hypothalamic-pituitary-gonadal (HPG) axis, as we have previously reviewed (Acevedo-Rodriguez *et al.* 2018). The execution of reproduction across most vertebrate species is dependent on hypothalamic neuropeptide/neurotransmitter production and release under the control of peripheral hormonal signals to regulate gametogenesis and mating. Gonadotropin-releasing hormone (GnRH) is the primary hypothalamic neuropeptide secreted in pulses that controls the release of pituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), from the anterior pituitary gonadotropes (Acevedo-Rodriguez *et al.* 2018). The gonadotropins, in turn, stimulate the gonads to produce steroids ,that is, testosterone (T), estrogens (primarily 17β-estradiol, E2), and progesterone (P4), and initiate gamete production. The gonadal steroids act as positive (only E2) and negative regulators of GnRH and gonadotropins by modulating neurons, including kisspeptin neurons, upstream of the HPG axis (Acevedo-Rodriguez *et al.* 2018), although neuro-progesterone produced by hypothalamic astrocytes has been implicated in the LH surge (Sinchak *et al.* 2020). Although many of the key pathways involved in the regulation of GnRH release are known, three neuronal populations, GnRH neurons and two kisspeptin neuron populations, act as an interconnected network to control the secretion of GnRH in pulses to temporally control the release of gonadotropins and the onset of puberty and the LH surge. Kisspeptin neurons, found in the anteroventral periventricular (AVPV) nucleus and the arcuate nucleus (ARC), have potent stimulatory actions on GnRH secretion and pulsatility and are also

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responsive to metabolic and physiological stressors to suppress reproduction (Acevedo-Rodriguez *et al.* 2018). AVPV kisspeptin neurons in rodents control the proestrus LH surge in females and contribute to the basal secretion of GnRH. ARC kisspeptin neurons co-express neurokinin B and dynorphin (called KNDy neurons) and are the GnRH pulse generator during most of the estrous cycle producing the tonic release of GnRH and LH. All of these neurons and the pituitary gonadotropes express at least one subtype of estrogen receptors (ER) and/or androgen receptors (AR). As such, each level of the HPG axis is tightly regulated and modulated by hormonal and central signals to influence reproductive status (Fig. 1 for illustration).

These hypothalamic neurons and pituitary cells are sensitive to endocrine influences during fetal, neonatal, and juvenile development and are controlled by the same hormone signals in the adult (Tena-Sempere 2010, García-Galiano *et al.* 2012). Thus, they are important targets for anthropogenic compounds which are known to interact with steroid (ER, AR) and nuclear receptors (aryl hydrocarbon receptor (AhR)) expressed by hypothalamic neurons and pituitary gonadotropes. These compounds, called endocrine-disrupting compounds (EDCs), exert

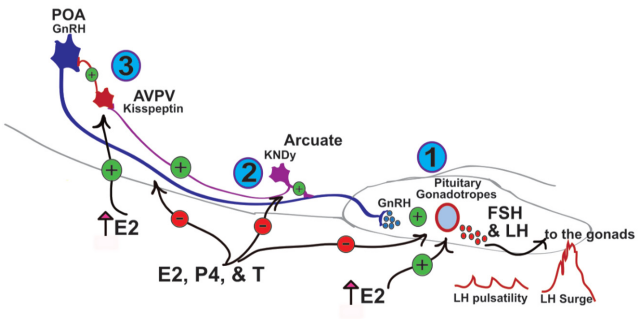

Figure 1 Modulation of GnRH and kisspeptin neurons by steroids controls gonadotropin production in the rodent. (1) The gonadal steroids, 17β-estradiol (E2), progesterone (P4), and testosterone (T) control the HPG axis through negative feedback onto hypothalamic neurons and pituitary gonadotropes. During negative feedback when progesterone levels are also elevated, E2 exerts a negative influence on GnRH (blue) pulsatility. Testosterone exerts negative feedback onto kisspeptin neurons and gonadotropes. GnRH is released from the nerve terminals in the median eminence into the primary porta plexus to activate the production of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). (2) GnRH pulsatility is controlled primarily through arcuate KNDy (purple) neurons. Kisspeptin is released onto GnRH terminals in the median eminence. The release of kisspeptin is controlled, in part, by the positive influence of neurokinin B (NKB) via Tac3 receptors expressed on neighboring KNDy neurons and by the negative influence of dynorphin release onto neighboring KNDy neurons or unidentified arcuate neurons that suppress kisspeptin release from KNDy neurons. (3) During positive feedback, elevated E2 plasma concentrations augment AVPV Kiss1 (red) neuronal activity and kisspeptin gene expression to produce the surge of GnRH and subsequently LH. Green circles (+) denote positive feedback or neuronal activation. Red circles (–) denote negative feedback or neuronal suppression.

their disruptive effects through multiple mechanisms as reviewed earlier (Waye & Trudeau 2011, La Merrill *et al.* 2020), which include: (1) acting as agonists and antagonists of hormone receptors, (2) altering hormone receptor expression and hormone signaling, and (3) disrupting hormone production, distribution, transport, and clearance. Exposure to common EDCs occurs throughout the lifespan of humans and wildlife as many EDCs are environmentally persistent and accumulate in lipid-rich tissues in the body (León-Olea *et al.* 2014, La Merrill *et al.* 2020).

One of the most well-known and well-studied EDCs is bisphenol A (BPA). Previous reviews have examined the evidence that this common EDC is a potential threat to reproduction through its interactions with GnRH, kisspeptin, and the pituitary (Tena-Sempere 2010, Huo *et al.* 2015, Santoro *et al.* 2019, Pivonello *et al.* 2020). Therefore, we will not be reviewing BPA but will focus on other well-known or emerging, but understudied, EDCs – dioxin and PCBs, organochlorine pesticides (DDT and methoxychlor (MXC)), perfluorinated alkyl substances (PFAS), and flame retardants. While many studies examining the influence of EDCs on the HPG axis have focused on the regulation of steroidogenic enzymes in the gonads and the production of steroid hormones (Waye & Trudeau 2011, La Merrill *et al.* 2020), our review will focus solely on the influences of these EDCs on hypothalamic GnRH and kisspeptin expression, GnRH pulsatility, and pituitary gonadotropin production from the pituitary and summarize their impact on the HPG axis. We will focus our discussion on rats and mice exposed perinatally or as juveniles and adults and briefly review exposure to hypothalamic or pituitary cell cultures for a comprehensive, yet not exhaustive, assessment of their influence on the HPG axis. We will also attempt to relate these findings to human physiology and highlight areas that demand further investigation.

Endocrine disrupting compounds

Humans are chronically exposed to dioxins, namely 2,3,7,8-tetrachlorodibenzodioxin (TCDD) and polychlorinated biphenyls (PCBs), which are a class of ~200 related compounds. Only co-planar PCBs act through similar mechanisms as dioxin, which is a potent agonist for the aryl hydrocarbon receptor (AhR) (Pessah *et al.* 2010, Larsson *et al.* 2015). Dioxins and PCBs are highly persistent in the environment and are known to bioaccumulate in animal and human adipose tissue leading to biomagnification in predators and human food sources (Schecter *et al.* 2006, McIntyre & Beauchamp 2007). Dioxins can occur naturally through combustion whereas anthropogenic sources occur through industrial manufacturing of paper, pesticides, herbicides, and electronics and through waste incineration leading to exposures greater than the tolerable weekly intake of 2 pg toxic equivalents (TEQ)/kg bw/week (Gilpin *et al.* 2003,

Fernandes & Falandysz 2021). While PCBs have been banned in the USA since 1979 and globally since 2001 (White & Birnbaum 2009), human exposure persists in the 1–300 pg/g lipid range due to the leaching of PCBs from old construction materials and hazardous waste sites (Consonni *et al.* 2012, Koh *et al.* 2015) with a no-observed-adverse-effect level (NOAEL) of 6–9 mg/kg/day (Graceli *et al.* 2020). Although their main mechanism of toxicity is through AhR activation, expressed in the hypothalamus and pituitary gonadotropes (Cao *et al.* 2011), many PCBs and dioxins can interact with steroid receptors or modulate their expression to exert EDC-like effects (Dickerson & Gore 2007, Akinola *et al.* 2021). Thus, the HPG axis is a target for these compounds as has been previously reviewed (Dickerson & Gore 2007). Our review focuses specifically on the kisspeptin-GnRH neural circuit and the pituitary and will discuss TCDD and PCBs.

Another class of chemicals that are classified as EDCs are organochlorine pesticides. Widely used since the middle of the 20th century, this class of pesticides includes DDT (1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane) and methoxychlor (1,1,1-trichloro-2,2-bis(p-methoxyphenyl)ethane, MXC). The metabolites of DDT and MXC are well-known EDCs (Tiemann 2008, Gore *et al.* 2011). Organochlorine pesticides and their metabolites possess estrogenic properties; resulting in adverse effects on the reproductive system in animal models (Cummings 1997, Martyniuk *et al.* 2020). In particular, DDT and its primary metabolites (DDE and DDD) are ubiquitous, persistent in the environment, bioaccumulate in lipid-rich organs and are detected in human samples (plasma 100–300 ng/g lipid) across the globe (Turusov *et al.* 2002, Rodríguez-Alcalá *et al.* 2015) with a NOAEL of 0.05 mg/kg/day and total daily intake (TDI) of 0.5 ng/kg/day (Graceli *et al.* 2020). Although DDT has not been in wide use in the USA and Canada since the early 1970s, exposures persist due to global transportation (Wu *et al.* 2020). MXC was developed as a replacement insecticide for DTT, but ultimately became obsolete and is no longer used. MXC has little endocrine activity and a low affinity for ERs. However, its primary metabolite HPTE (2,2,-bis-(*p*-hydroxyphenyl)-1,1,1-tric hloroethane) has a high affinity for ERs and thus has become a model estrogenic EDC (Cummings 1997). The influence of DTT and MXC on female fertility, ovarian function, and implantation in mammals was previously reviewed (Tiemann 2008). Thus, we are focusing on the hypothalamic and pituitary impacts of these EDCs on the reproductive axis in rodents and cell cultures.

PFAS are synthetic chemicals that are insoluble in water and solvents, while also repelling water and oils. Thus, PFAS are used for both household and industrial applications from shampoos to cookware and insecticides to fire-retardant foams. Ingestion via food, water, or food packaging and preparation is the main route of exposure in human populations (Domingo & Nadal 2017). Common PFAS, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), are detected in human samples (blood, urine) from infants, children, and pregnant and nursing mothers at 1–25 ng/mL and are environmentally persistent, ubiquitous, and bioaccumulate in humans and wildlife (Ballesteros *et al.* 2017, Liu *et al.* 2018). PFAS have disruptive properties to the hypothalamic-pituitary-thyroid axis that play an important neurodevelopmental role *in utero* and neonatal neuromaturation to adolescence and adulthood (Coperchini *et al.* 2017). In regards to reproduction, data from maternal PFOA exposure studies in rodents suggest that PFOA disrupts lactational efficiency in the dams leading to offspring mortality, delayed mammary development, and altered mammary gene expression (Patisaul *et al.* 2018). Furthermore, peripubertal PFOA exposure in female mice disrupts estrous cyclicity and genes involved in ovarian steroidogenesis in a strain-dependent manner (Zhao *et al.* 2012). It is only within the past decade that more attention has been focused on the effects of PFAS on the HPG axis in mammals.

Flame retardants are compounds added or adhered to furniture, clothing, toys, electronics, plastics, and other household and workplace furnishings to reduce combustibility to meet government fire safety regulations. Flame retardants are often volatile and are not chemically bound to the fabric, plastic, or materials. Flame retardants are typically found in house dust, food, and water and in the air of offices, airplanes, and other workplace environments (Xue *et al.* 2007, Hoffman *et al.* 2017, Wong & Durrani 2017, Gravel *et al.* 2020, Percy *et al.* 2020). There are three main groups: polybrominated diphenyl ethers (PBDEs), novel brominated compounds, and organophosphate flame retardants (OPFRs). PBDEs have been largely phased out of use since the mid-2000s due to their bioaccumulation in humans and wildlife, and their neurological and endocrine toxicity (Costa & Giordano 2007, van der Veen & de Boer 2012, Hu *et al.* 2014, Abbasi *et al.* 2015). PBDEs have been replaced by novel brominated compounds and OPFRs (van der Veen & de Boer 2012), which are not as persistent and bioaccumulate less than PBDEs (Yang *et al.* 2019). PBDEs were detected in human samples (placental and breast milk), 5–10 years after the phase-out, at concentrations ranging from 1 to 60 ng/g lipid (Adgent *et al.* 2014, Leonetti *et al.* 2016, Eskenazi *et al.* 2017) and standard safety levels set at 0.1–7 µg/kg/day (Graceli *et al.* 2020). Body burdens of OPFRs and their metabolites are increasing in human populations, with some metabolites found at levels 15 times higher in 2014–2015 than in 2002–2003 (Hoffman *et al.* 2017, 2018, Blum *et al.* 2019). For example, OPFRs are found in household dust at median concentrations of ~1–6 µg/g dust (triphenyl phosphate (TPP), tricresyl phosphate (TCP), and tris(1,3-dichloro-2-propyl)phosphate (TDCPP)), which are inadvertently ingested by a pregnant mother or a growing infant (Carignan *et al.* 2013, Meeker *et al.* 2013, Fan *et al.*

2014). Consequently, OPFR metabolites are detectable in humans during the perinatal period (Hoffman *et al.* 2015) in urine, breast milk, and blood samples ranging in concentrations from 1 to 100 ng/mL and 1 to 100 ng/g lipids (Sundkvist *et al.* 2010, Butt *et al.* 2014, Dishaw *et al.* 2014, Hoffman *et al.* 2014, 2017, Kim *et al.* 2014). While OPFR exposures are associated with increased risk for high BMI in children (Boyle *et al.* 2019) and adverse neurological outcomes (Castorina *et al.* 2017, Doherty *et al.* 2019), little is known about their influence on the hypothalamus or pituitary. Summary Tables (Tables 1, 2, 3 and 4) are provided for each group of EDCs.

Perinatal exposures

The perinatal period, which encompasses gestation and lactation periods and fetal, neonatal, and pre-weaning juvenile developmental windows, is especially sensitive to the perturbations from EDC exposure. The primary animal model used in perinatal EDC exposures is the rat, although studies using mice are now common. These animal models are used to assess the influence of EDCs on maternal programming and early juvenile development, which correlates to similarly sensitive windows in human development. In regards to dioxins, the prenatal (fetal) stage is highly sensitive. For example, a single dose of TCDD (0.800 µg/kg) at GD15 in Long-Evans hooded rats advanced the onset of puberty (vaginal opening) and first estrus day while increasing ovarian weights (Kakeyama *et al.* 2008). A single dose of TCDD (1 µg/kg) at GD15 did not alter GnRH expression but reduced fetal (GD20) pituitary expression of LHβ and serum LH concentrations. The authors provide evidence that the decrease in serum T is due to a reduction in steroidogenic enzymes in particular StAR protein. The reduction was due to the decrease in LH production because StAR expression in the fetal testes was rescued from TCDD exposure via injection of equine chorionic gonadotropin (eCG) into the fetus at GD15 (Mutoh *et al.* 2006). In a study of hypothalamic explants from maternally exposed Sprague–Dawley male offspring (PND35), a single dose of TCDD (5 µg/kg, GD15) suppressed KCl-evoked release of GnRH to 40% of controls (Clements *et al.* 2009). Suppression of GnRH was also observed at PND60 and 90 indicating that TCDD has long-lasting effects beyond puberty on GnRH release. Not only was GnRH release altered by TCDD exposure but hypothalamic GnRH content and GnRH somal profiles (smooth, lightly thorny (spines), heavily thorny) were altered in the lateral POA with more smooth-type and fewer thorny-type GnRH neurons. Smoother cells suggest fewer presynaptic inputs and potentially less excitatory inputs onto GnRH neurons. These data suggest that single exposure to TCDD during fetal development can have long-lasting effects on the reproductive parameters of the offspring, albeit at doses higher than typical human exposure.

Perinatal TCDD exposure can also induce long-lasting disturbances to reproduction. TCDD exposure (50 or 200 ng/kg/week by gavage) every week for 4 weeks across the perinatal period (GD14, GD21, PND7, PND14) delayed the onset of puberty and suppressed E2 production throughout the cycle (Shi *et al.* 2007). In ovariectomized females from this experiment, TCDD exposure at 200 ng/kg/week potentiated the induction of LH by GnRH 1 h post-injection with no change in FSH production. These doses were designed to be within the range of human exposure, whether across

Table 1 Summary of effects of dioxin on GnRH, kisspeptin, and gonadotropins.

Source	Exposure	Dose	Model	GnRH and pulse	Kisspeptin	Gonadotropins
Kakeyama *et al.* (2008)	Perinatal	200 or 800 ng/kg	Rat			
Mutoh *et al.* (2006)	Perinatal	0.01, 0.1, 1 µg/kg	Rat	NC		F, M: ↓LHβ, serum LH
Shi *et al.* (2007)	Perinatal	1–200 ng/kg/week	Rat			F: ↑LH 1 h post-GnRH
Takeda *et al.* (2014)	Perinatal	1 µg/kg/2 mL vehicle	Rat	M: ↓		M: ↓LH
Clements *et al.* (2009)	Perinatal	5 µg/kg	Rat	M: ↓		
Takeda *et al.* (2011)	Perinatal	1 or 10 nM	Rat: PC			↓*Lh*β, α*Gsu*
Li *et al.* (1997)	Juvenile	10 µg/kg	Rat			F: ↑LH and FSH
Gao *et al.* (2000)	Juvenile	32 µg/kg	Rat			F: ↓LH and FSH
Petroff *et al.* (2003)	Juvenile	8 or 32 µg/kg	Rat			F: ↑FSH and LH
Bookstaff *et al.* (1990)	Adult	100 µg/kg	Rat	M:↓Gnrhr		M: ↓LH
Cao *et al.* (2011)	Adult	0.5 and 10 µg/kg	Rat			F: ↑*Lh*β
Yin *et al.* (2012)	Adult	100 ng/kg/day	Mouse	M:↓		M: ↓testicular LH, FSH
Trewin *et al.* (2007)	Adult	3.2 nM	Rat explants	NC		NC
Huang *et al.* (2011)	Transgeneration	0 and 2 ng/L	Mouse	NC		NC
Petroff *et al.* (2003)	Cell	1–100 nM	GT1-7	NC		
Solak *et al.* (2013)	Cell	10 nM	Rat: GnV-3	↑↓*Gnrh*		
Mueller & Heger (2014)	Cell	10 mM	GripTite™ 293 MSR cells transfected with the *Kiss1* promoter	↓*Gnrh*	↓*Kiss1*	

↑Increase in expression of gene or protein; ↓Decrease in gene or protein; ↑↓Mixed effects.
F, female; M, male; NC, measured but no change; PC, pituitary cells.

Table 2 Summary of effects of PCBs on GnRH, kisspeptin, and gonadotropins.

EDC	Source	Exposure	Dose	Model	GnRH and pulse	Kisspeptin	Gonadotropins
A1221; PCB138,153, 180	Dickerson *et al.* (2011*a*)	Perinatal GD16–18	1 mg/kg	Rat	F: ↓ no. of *c-fos*+ GnRH cells	F: ↓ no. of Erα^{+} AVPV neurons (kisspeptin?); ↓AVPV	
A1221; PCB138,153, 180	Dickerson *et al.* (2011*b*)	Perinatal PND1	1 mg/kg	Rat	NC	NC: *Kiss1*; M:↓Kiss1r; F:↑Kiss1r	
A1221	Steinberg *et al.* (2008)	Multigenerational	0.1–10 mg/kg	Rat			F: ↑serum LH in F1; ↓proestrus LH in F2
A1242	Jansen *et al.* (1993)	Adult – primary pituitary cultures	0.1–100 ppm	Rat			↑LH & FSH after GnRH-stimulation; ↓LH; ↓FSH w/ GnRH
PCB153	Desaulniers *et al.* (1999)	Young adult	25 mg/kg/day	Rat			↓serum LH; no effect on pituitary LH
PCB126	Han *et al.* (2010))	Young adult	0.2 mg/kg PCB126; 20 mg/kg of PCB114	Rat			M:↓ serum FSH PCB126; ↑serum LH PCB114
PCB114							
PCB126	Desaulniers *et al.* (1999)	Adult	6.25–400 mg/kg/day	Rat			M:↓ serum LH; ↑pituitary LH and FSH
PCB180A1221	Uslu *et al.* (2013)	Adult	10 mg/kg	Rat			F: ↓ serum FSH

↑Increase in expression of gene or protein; ↓Decrease in gene or protein.
F, female; M, male; NC, measured but no change.

the reproductive lifespan or in high-risk populations and occupational exposures. In another perinatal study supporting the long-term effects of TCDD exposure on adult GnRH activity, pregnant Wistar dams were orally dosed with TCDD (1 µg/kg per 2 mL of corn oil) at GD15 followed by sample collection at GD18, 19, 20, and 21 and PND0, 2, 4, 7, 14, 28, 56, and 70 (Takeda *et al.* 2014). TCDD exposure reduced hypothalamic GnRH expression in juvenile and young adult male offspring and reduced GnRH protein content in the hypothalamus, cerebrum, and cerebellum at PND70. However, GnRH mRNA was reduced in the hypothalamus across all postnatal ages, but only in the cerebrum or cerebellum at PND70. TCDD exposure also attenuated the fetal and neonatal expression of pituitary LH. Furthermore, adult (PND70) male offspring exposed to TCDD during gestation exhibited reduced mounting and intromissions when administered GnRH. Although testosterone was not measured, these effects on sexual behavior may be due to reduced steroidogenesis during development as StAR protein and 17α-hydroxylase (Cyp17) expression was reduced in fetuses and neonates suggesting that the organizational effects of TCDD endocrine disruption in the brain underlie the deficits in sexual behavior. In a transgenerational mouse study, TCDD was administered via drinking water at 2 ng/L (2 ppt) to C57 BL/6J females starting at weaning (PND21). Dams were later mated and their offspring were studied over the next two generations (Huang *et al.* 2011). TCDD exposure reduced E2 levels in young adult females in the P0 and P1 generations and this was associated with a higher incidence of miscarriage and stillbirth with no change in P4. These parameters are an indirect measurement of HPG activity/reproductive toxicity suggesting disruption of the HPG axis by TCDD at concentrations not exceeding exposure limits to humans.

PCBs are another EDC that can interact with both AhRs and steroid receptors as either parent compound or their metabolites. Only a few studies illustrating the potential disruption to GnRH, kisspeptin, and gonadotropin production due to perinatal PCB exposure exist. In two similar studies from the same laboratory, exposure of pregnant Sprague–Dawley dams to an estrogenic-positive control (estradiol benzoate (EB), 50 µg/kg), Aroclor 1221 (A1221, 1 mg/kg), or a mixture of nonplanar PCBs with the highest body burden in humans (PCB 138, 153, 180, at 1 mg/kg) on GD16 and 18 increased anogenital distance (AGD) in male pups (PND1) and reduced AGD in female pups (Dickerson *et al.* 2011*a*). A1221 increased the number of apoptotic nuclei in the female AVPV, but not the medial preoptic area (MPOA). When POA gene expression from PND1 pups was examined by low-density PCR arrays, *Kiss1* and *Gnrh* expression were not affected by the EDCs but kisspeptin receptor was reduced in males, yet increased in female pups (Dickerson *et al.* 2011*a*). In the second study using the same EDCs and experimental design but focusing on pups, juveniles,

Table 3 Summary of effects of organochlorine pesticides on GnRH, kisspeptin, and gonadotropins.

EDC	Source	Exposure	Dose	Model	GnRH and pulse	Kisspeptin	Gonadotropin
DDE	Makita (2008)	Perinatal	125 ppm	Rat			F: NC
MXC	Roepke *et al.* (2016)	Perinatal		F rat		NC ARC	
MXC	Masutomi *et al.* (2004)	Perinatal	1200 ppm	Rat			M: ↓% of LH^+, FSH^+ cells; F: ↓LH^+ cells, ↑% of FSH^+ cells
MXC	Suzuki *et al.* (2004)	Perinatal	24, 240, 1200 ppm	M rat			M: ↓serum FSH, LH; F: ↓proestrus LH surge
o,p′-DDT, p,p′-DDE	Rasier *et al.* (2007)	Juvenile	10, 100 mg/kg/day	F rat	↓GnRH IPI; ↑pulsatility in PND15		↓serum LH concentrations
o,p′-DDT p,p′-DDT	Rasier *et al.* (2008)	Juvenile	10^{-5}–10^{-4} M	F rat	↑glut-evoked GnRH, pulse amp, pulsatile GnRH		
MXC	Dickerson *et al.* (2011*b*)	Juvenile	20 µg/kg, 100 mg/kg	F rat		NC AVPV	
DDT	Ben Rhouma *et al.* (2001)	Adult	50 mg, 100 mg/kg	Rat			M: ↑FSH, LH
MXC	Lafuente *et al.* (2008)	Adult	25 mg/kg/day	Rat			↓plasma LH; NC FSH
MXC	Tomic *et al.* (2006)	Adult	64 mg/kg/day	Mouse			↓serum FSH levels
p,p′-DDT MXC	Zhou *et al.* (2014)	Cell	10^{-7} M	LβT2 cells			↑stimulation *Fshβ*, *Lhβ*, FSH, LH protein

↑Increase in expression of gene or protein; ↓Decrease in gene or protein.
F, female, M, male; NC, measured but no change.

and young adult offspring, exposure to A1221, the PCB mixture, or EB increased anogenital distance in male pups from PND1 to 21, while only A1221 increased body weights in juvenile and adult female offspring (Dickerson *et al.* 2011*b*). All three treatments induced early onset of puberty in female offspring but delayed puberty (preputial separation) in male pups. These exposures reduced the number of ERα-expressing AVPV neurons (potentially kisspeptin) and the volume of the AVPV in adult female offspring. A1221 and PCBs also reduced AVPV kisspeptin expression and the number of GnRH neurons expressing *c-fos*, an early immediate gene indicating neuronal activation, on the afternoon of proestrus. The authors also reported main effects of PCBs on serum T and main effects of A1221 and PCBs on serum P4 in males (Dickerson *et al.* 2011*b*). In a multigenerational perinatal study, pregnant Sprague–Dawley rats were dosed with A1221 (0.1, 1, or 10 mg/kg) on GD16 and 18 (Steinberg *et al.* 2008). In the F1 generation, the A1221 treatment did not alter serum P4 and E2 but increased serum LH concentrations. In the F2 generation, all doses of A1221 reduced P4 during proestrus, and the high dose reduced P4 during diestrus. The high dose reduced F2 uterine weights during estrus and LH during proestrus. These three studies strongly suggest that disruption of GnRH and kisspeptin or its receptor by PCBs in the hypothalamus may lead to long-term disruption of the HPG axis that can extend through generations of female rats.

Table 4 Summary of effects of PFAS on GnRH, kisspeptin, and gonadotropins.

EDC	Source	Exposure	Dose, mg/kg	Model	GnRH and pulse	Kisspeptin	Gonadotropins
PFOA	Du *et al.* (2019)	Perinatal (PND: 1–5)	0.1, 1,10	Rat		F: ↓ AVPV; ↓ ARC *Kiss1*, *Kiss1r*	F: ↑ LH serum in high dose
PFOA	Du *et al.* (2019)	Juvenile (PND: 26–30)	0.1, 1, 10	Rat		F: ↑ AVPV low dose; ↓ AVPV high dose	F: ↑ LH in high dose
PFOS	López-Doval *et al.* (2014)	Young adult	0.5, 1.0, 3.0, 6.0	Rat	M: ↓ at 3 low doses		M: ↑ LH, FSH at 2 low doses M: ↓ LH at 0.5 and 2.0 kg M: ↑ FSH all doses
PFOS	López-Doval *et al.* (2016)	Adult	1.0, 3.0, 6.0	Rat	M: NC: *Gnrh*		M: NC - *Gnrhr* M: ↓ GnRH receptor protein
PFOS	Feng *et al.* (2015)	Adult	0.1	Mouse	F: ↑ in diestrus F: ↓ in proestrus	F: ↓ AVPV $Kiss^+$ neurons F: ↓ AVPV	F: ↓ LH, FSH proestrus F: ↑ LH diestrus
PFAS	Zhang *et al.* (2020)	Adult	0.5, 2, 5	Mouse	↓ at 2 and 5 mg/kg		↓ LH at 2 and 5 mg/kg

↑Increase in expression of gene or protein. ↓Decrease in gene or protein.
F, female; M, male; NC, measured but no change.

Epidemiological studies have demonstrated associations between DDT or its metabolites (DDE, DDD, etc.) and preterm and undersized neonates with a range of DDE from 3 to 178 ng/mL (Longnecker *et al.* 2001). While there are numerous studies on DDT and reproduction in rodent models, there are only a few that measure the activity of the HPG axis after perinatal DDT exposure. In a maternal exposure study using Wistar rat dams, DDE exposure (via food, 125 ppm, from GD1- PND21) did not alter the onset of puberty, estrous cyclicity, or serum levels of FSH and LH in young female offspring (Makita 2008). In a postnatal exposure model, young female rats were exposed to the o,p′-DDT isomer or its metabolite, p,p′-DDE, during postnatal development (PND6 onward), followed by measurements of *in vitro* GnRH secretion and the onset of puberty and gonadotropin secretion (Rasier *et al.* 2007). In hypothalamic explants from juvenile females (PND15), o,p′-DDT and p,p′-DDT (10^{-6} to 10^{-4} M) reduced GnRH interpulse intervals (increased pulsatility) after 3–4 h in a concentration- and time-dependent manner with no effect on amplitude. In an *in vivo* experiment, female rats received daily s.c. injections of o,p′-DDT for 5, 10, or 35 days (10 or 100 mg/kg/day for PND6–10 and 10 mg/kg/day for PND6–15 and PND6-40). In PND15 female rats, serum LH concentrations were reduced, potentially through a negative feedback effect directly on gonadotropes during the pre-pubertal period, as the authors suggest. However, GnRH pulsatility was increased (lower interpulse intervals) after 10 and 100 mg/kg o,p′-DDT, which correlated with early onset of puberty (vaginal opening) and first estrus. *In vitro* exposure of hypothalamic explants collected from PND15 female pups to o,p′-DDT and p,p′-DDT (10^{-5} to 10^{-4} M) potentiated glutamate-evoked GnRH secretion by increasing the amplitude of pulses (Rasier *et al.* 2008). *In vivo* exposure to o,p′-DDT (10 mg/kg/day, PND6–10) accelerated pulsatile GnRH secretion and induced early onset of puberty in juvenile female rats. Antagonists to both ERα and AhR blocked the effects of o,p′-DDT on GnRH pulsatility. These studies are unique in that they analyze the effect of short-term exposure to a high dose estrogenic EDC on GnRH pulsatility, on both amplitude and frequency. These data also indicate transient exposure to doses of DDT higher than body burdens in pregnant women (Longnecker *et al.* 2001) that alters GnRH pulsatility leading to early sexual maturation.

While MXC is no longer in use and human exposures are minimal, this EDC and its strongly estrogenic metabolite HPTE (Hewitt & Korach 2011) are useful models for reproductive disruption by estrogenic EDCs. Perinatal exposures to MXC in rodents disrupt reproductive parameters (puberty) and hypothalamic gene expression and pituitary gonadotropin production. In Fischer rats, aged female offspring exposed to daily injections of MXC (20 µg/kg or 100 mg/kg) from GD19 to PND7 through the dams exhibited reduced estrous cyclicity by 14 months of age compared to DMSO control, and the high dose of MXC induced complete anestrus by 17 months while all DMSO controls were still cycling (Gore *et al.* 2011). These effects on cyclicity were associated with lower serum E2 and elevated *Esr1* (ERα) and *Gnrhr* expression in the POA, with no effect on POA *Kiss1*. In a similar perinatal exposure study of Fischer rats, female adult offspring from dams exposed to MXC (75 mg/kg/day from GD11 to PND7) exhibited an early onset of puberty (vaginal opening), but no effect on *Kiss1* expression in the arcuate nucleus (Roepke *et al.* 2016). In another maternal exposure study, MXC (1200 ppm) was introduced via a special diet from GD15 to PND10 in an unidentified rat strain. The MXC-containing diet reduced the percentage of LH^+, FSH^+, and $prolactin^+$ cells in male juvenile offspring (PND21) and reduced LH^+ cells in juvenile females (PND21). In the young adult offspring, MXC exposure increased the percentage of FSH^+ and $prolactin^+$ cells only in female offspring to 134% of control (Masutomi *et al.* 2004). In another perinatal exposure model using Wistar-Imamichi rats, MXC exposure via diet (24, 240, 1200 ppm, GD15 - PND10) decreased serum FSH and LH, but not T, after maturation in male offspring (Suzuki *et al.* 2004). In female offspring, the lordosis quotient was reduced by all doses of MXC which was associated with reduced proestrus LH surge concentrations in serum. Collectively, these data suggest that high doses of MXC during the perinatal period disrupt the HPG axis in both female and male rats.

While at doses higher than recently reported PFAS concentrations in human samples (Ballesteros *et al.* 2017, Liu *et al.* 2018), PFAS exposure appears to specifically target the steroid-sensitive kisspeptin neurons in juveniles that lead to changes in sexual maturation. In rats, the response to PFAS is dependent on the duration and dose of exposure, the developmental window of exposure, and the age and sex of the animal. In a recent study using neonatal and juvenile female Sprague–Dawley rats, s.c. injection of PFOA (0.1, 1, and 10 mg/kg) or PFOS (0.1, 1, and 10 mg/kg) during PND1–5 suppressed *Kiss1* and *Kiss1r* mRNA expression in the AVPV and the ARC in the postpubertal adult rats compared to controls (Du *et al.* 2019). Conversely, in the juvenile exposure on PND 26–30 to a low dose of PFOA (0.1 mg/kg) increased AVPV *Kiss1* expression whereas a high dose (10 mg/kg) decreased AVPV *Kiss1*. These effects on kisspeptin expression were associated with elevated kisspeptin fiber intensities at the low doses and suppressed kisspeptin fiber intensities at the high doses in the AVPV and ARC. Physiologically, these central effects correlated with elevated LH serum concentrations in neonates and an advancement of puberty onset (vaginal opening) and first estrus at 10 mg/kg for both neonatal and juvenile exposures.

There are few studies that examine the effects of flame retardants on GnRH, kisspeptin, and pituitary production

of gonadotropins in rat perinatal or adult exposures. In one study on the maternal exposure (GD10–18) of rats to PBDE 99 (1 and 10 mg/kg/day), more exposed adult female offspring exhibited irregular estrous cycles than controls (Faass *et al.* 2013). While GnRH, kisspeptin, or gonadotropins were not assessed, PBDE 99 exposure increased ERα expression in the MPOA and the ventromedial hypothalamus (VMH) while suppressing VMH ERβ and progesterone receptor (PR). As these two regions are important for sex behavior, these results indicate that PBDEs could disrupt the hormonal control of sexual motivation and lordosis. In male rats, VMH PR mRNA induction in response to E2 was reduced by 1 mg PBDE 99, but increased by 10 mg (Faass *et al.* 2013). In a recent mouse study from our lab, maternal exposure to a mixture of OPFRs (TPP, TCP, TDCPP; 1 mg/kg/day from GD7 to PND14) induced transcriptional changes to several reproductive hypothalamic genes in neonatal (PND0) and juvenile (PND14) WT C57 pups (Adams *et al.* 2020). In female juvenile pups, the expression of *Pdyn*, *Tac2*, and *Esr1* was elevated by maternal exposure to OPFRs. These changes are associated with an increase in body weight at PND14 and a decrease in the anogenital distance in male pups at PND7, indicating an estrogenic effect of OPFR exposure or inhibition of the neonatal testosterone surge. Collectively, these few studies suggest that developmental exposures to PBDEs or OPFRs can disrupt the hypothalamic expression of neuropeptides and steroid receptors. These findings warrant further investigation with particular attention on the mechanism of toxicity and the subsequent effects in pre- and post-pubertal rodents.

Adult exposures

Exposures to EDCs are continuous and occur across the lifespan of an individual. However, most adult studies of EDC on the HPG axis in rodents do not use continuous lifelong exposures but focus on single dose or sub-chronic exposures to doses higher than human exposures. For example, exposures to TCDD at high doses influence hypothalamic and pituitary reproductive endpoints in young and adult rats. In a 1990 study, a single high dose of TCDD (100 µg/kg, oral) given to adult Sprague–Dawley male rats reduced plasma T, prevented the compensatory increase in plasma LH, and blocked the increase in GnRH receptors in the pituitary (Bookstaff *et al.* 1990). However, in castrated male rats, TCDD did not affect LH or GnRH plasma concentration or pituitary GnRH receptor expression suggesting that TCDD potentiates the negative feedback of androgens on the hypothalamus and pituitary. In immature (PND22-23), female Sprague–Dawley rats, TCDD injection can alter gonadotropin production in a dose- and time-dependent manner. A single dose of TCDD (10 µg/kg) by gastric intubation elicited a distinct increase in both LH and FSH serum concentration about 24 h after dosing that was not observed in the controls (Li *et al.* 1997). FSH production was more sensitive to TCDD as FSH was induced at 0.3, 1, 3, 10, and 30 µg/kg while LH was induced at 3, 10, and 30 µg/kg. Collectively, these data suggest that a single high dose TCDD is estrogenic as it potentiates the release of gonadotropins in females and by reducing androgen production in male rats. In another study using immature female rats, TCDD (32 µg/kg) injected alone reduced LH and FSH production 58 h after an injection of equine chorion gonadotropin (eCG) (which induces folliculogenesis) and significantly reduced the number of ova collected in the oviduct compared to control. However, TCCD injection did not alter the induction of LH or FSH when administered with GnRH suggesting that the disruption to ovulation is a direct effect on the ovary (Gao *et al.* 2000). In a third study using immature female Sprague–Dawley rats, TCDD (8 or 32 µg/kg orally) increased FSH and LH serum levels prematurely after eCG injection (Petroff *et al.* 2003). Furthermore, TCDD (32 µg/kg) increased serum LH and FSH compared to control when estradiol cypionate was also administered. In adult ovariectomized female Sprague–Dawley rats, TCDD at 0.5 and 10 µg/kg increased pituitary *Lhb* expression, which was suppressed by E2 (Cao *et al.* 2011). In an adult subchronic exposure model using an unidentified mouse model, oral TCDD exposure at a low dose (100 ng/kg/day for 7 weeks) reduced hypothalamic GnRH protein expression and testicular LH and FSH content in males (Yin *et al.* 2012). The disruptive effects of subchronic TCDD exposure were partially ameliorated by vitamin E supplementation by reducing oxidative stress and expression of AhR target genes (Hamm *et al.* 2000, Xu *et al.* 2008). These data indicate that TCDD may have age- and sex-dependent effects on GnRH actions in the pituitary and on gonadotropin production, as well as disruption to gonadal function through direct and indirect pathways. Thus, TCDD has the capability of disrupting the mouse HPG axis both at single high-dose exposure or subchronic low-dose exposures, although more investigations are needed.

In experiments focusing on adult PCB, DDT, and MXC exposures, both gonadotropin and steroid production are especially sensitive. In adult male Sprague–Dawley rats, PCB 126 injections (6.25, 25, 100, or 400 µg/kg/day for 2 days) reduced serum LH but increased pituitary LH and FSH, indicating that PCB 126 suppresses the release of LH, driven by GnRH, but not to the same degree as synthesis (Desaulniers *et al.* 1999). In another acute PCB exposure study in young adult male Sprague–Dawley rats, weekly injections of PCB126 (0.2 mg/kg) or PCB114 (20 mg/kg) for 2, 5, or 8 weeks altered serum T and LH (Han *et al.* 2010). In the 2-week PCB 126 and PCB 114 groups, serum T was suppressed, and in the 5-week PCB126 group, serum T was also lower than controls, due to a reduction in the expression of steroidogenic enzymes in the testes. Consequently,

serum LH in the 5-week PCB114 group was elevated, and serum FSH in the 2- and 5-week PCB126 groups were lower compared to controls. Potentially many of the effects observed in PCB exposure are exerted in the gonads leading to suppression of the negative feedback signal (T) that in turn increases LH production. These effects may also be dependent on the sex or the strain of the rat. For example, in female Wistar rats subcutaneously injected with either PCB 180 or A1221 (10 mg/kg for 3 days), serum FSH was suppressed but not serum LH (Uslu *et al.* 2013). Exposure to DDT (50 and 100 mg/kg for 10 days) in adult male rats severely diminished serum T in a dose-dependent manner (Ben Rhouma *et al.* 2001). Subsequently, FSH levels were elevated at both doses and LH levels at 100 mg/kg due to the lack of negative feedback from testosterone. In an adult exposure paradigm in Sprague–Dawley rats, MXC (s.c. injection, 25 mg/kg/day for 30 days) decreased plasma LH and T as compared to control with no effect on FSH (Lafuente *et al.* 2008). In ERα-overexpressing mice (tet-op-tTA/tet-op-luciferase mice/tet-op-ERα), MXC (i.p. injection, 64 mg/kg/day for 20 days) increased time spent in estrus in both littermate controls and ERα-overexpressing mice, and overall showed reduced serum FSH levels, with no effect on E2 although greater atresia of ovarian follicles was observed in the ERα-overexpressing mice (Tomic *et al.* 2006). This last study is one of the few studies that investigated a hormone receptor-mediated mechanism of HPG axis disruption in a rodent model. Collectively, these adult exposure studies to EDCs illustrate the importance of examining the entire HPG axis to determine the site of endocrine disruption as disruption to steroidogenesis in the gonads produces effects at the level of hypothalamus and/or pituitary hormone production.

An emerging threat to reproductive health is the pervasive EDC class – PFAS. In male Sprague–Dawley rats, subchronic oral exposures to PFOS (0.5, 1.0, 3.0. and 6.0 mg/kg/day for 28 days) administered during young adulthood (PND60), reduced hypothalamic GnRH expression at the three lowest doses, increased pituitary LH and FSH expression at 0.5 and 1.0 mg/kg, and decreased circulating LH levels in 0.5 and 3.0 mg/kg/day groups (López-Doval *et al.* 2014). However, all animals exposed to PFOS exhibited an increase in serum FSH. In a follow-up study from the same group, adult PFOS exposure increased serotonin concentrations in the anterior and mediobasal hypothalamus and the median eminence, suppressed hypothalamic neuropeptide Y expression, and increased expression of genes involved in nitric oxide signaling and metabolism (López-Doval *et al.* 2015). Interestingly, noradrenaline concentrations increased in the anterior hypothalamus and the median eminence, where the neurotransmitter likely stimulates GnRH and kisspeptin neuronal activity (Kalil *et al.* 2016, Terasawa 2019). Since these three signals (noradrenaline, serotonin, nitric oxide) stimulate GnRH neurons and have a role in modulating GnRH release, the modulation of these signals by PFOS exposure suggests that adult exposure to these compounds may contribute to HPG axis dysregulation and infertility. In a final study from the same lab, PFOS exposure (1.0, 3.0, and 6.0 mg/kg/day for 28 days, oral gavage) in adult male rats did not modify pituitary GnRH receptor gene expression but suppressed receptor protein expression at all doses (López-Doval *et al.* 2016). Thus, high doses of PFOS administered to adults disrupt the expression of hypothalamic neuropeptide expression, neurotransmitter release, and pituitary gonadotropin production. This disruption may negatively influence estrous cyclicity. Indeed, injection of PFOS at 1 and 10 mg/kg/day for 14 days suppressed estrous cyclicity in adult female rats inducing irregular cycles or persistent estrus in 20–30% of the females. Females exposed to 10 mg/kg also exhibited elevated norepinephrine in the paraventricular hypothalamus but not the MPOA which correlated with elevated plasma corticosterone, suggesting that disruption of the HPA axis underlies the effects on estrous cyclicity (Austin *et al.* 2003).

In mice, studies on PFAS exposure and the hypothalamus have focused solely on female mice. In a chronic (6 months) PFOS exposure model via drinking water (0.1 mg/kg/day) in adult female ICR mice, POA GnRH mRNA expression was reduced by PFOS during proestrus but elevated during diestrus, which correlated with similar changes in hypothalamic GnRH protein content (Feng *et al.* 2015). Chronic PFOS exposure also modified LH and FSH dependent upon the stage of the estrous cycle. LH and FSH were reduced during proestrus, but only LH was elevated by PFOS during diestrus. These data correlated with a suppression in the number of AVPV $Kiss^+$ neurons and AVPV *Kiss1* expression. PFOS also reduced E2 serum concentrations suggesting that PFOS disrupts the E2-induced activation of AVPV kisspeptin neurons necessary for the generation of the LH surge. Indeed, PFOS extended the diestrus stage by 1 day and impaired the LH surge during proestrus, although not the sensitivity of LH to the positive feedback of E2 or kisspeptin injection. In a follow-up study from the same laboratory, subchronic exposure to PFOS (7–30 days, 10 mg/kg, oral) in adult female ICR mice reduced serum concentrations of LH and GnRH after 7 and 14 days of exposure and nearly tripled the length of diestrus, producing irregular cyclicity (Wang *et al.* 2018). PFOS exposure also reduced the number of *Kiss1*+ AVPV neurons and *Kiss1* protein and gene expression during proestrus without altering ARC *Kiss1*. Furthermore, PFOS suppressed the E2-induced increase in AVPV *Kiss1* mRNA and protein expression and the E2-induced hypothalamic GnRH content and the surge in LH. In a recent study from the same laboratory, another PFAS, PFOA, altered the HPG axis and hypothalamic kisspeptin expression in adult (12 weeks) ICR female mice (Zhang *et al.* 2020). PFOA,

administered by gavage (0.5, 2, or 5 mg/kg/day for 28 days), reduced GnRH and LH serum concentrations at 2 and 5 mg/kg/day. PFOA suppressed the endogenous LH surge and the E2-induced LH surge potentially by attenuating the expression of AVPV *Kiss1* mRNA through elevated liver FGF21 production (Zhang *et al.* 2020). FGF21 was recently demonstrated to suppress actions of vasopressin from the suprachiasmatic nucleus in activating AVPV kisspeptin during the proestrus surge of LH (Owen *et al.* 2013). Collectively, these data suggest that exposure to high doses of PFAS compounds influences the hypothalamic neurons that control the HPG (and the HPA axis), at the level of gene and protein expression, leading to dysregulation of reproduction. Determining the effects of environmentally relevant doses of these PFAS/PFOS on these genes and the HPG axis in a rodent model should be investigated further.

The influence of adult exposures to flame retardants (PBDEs or OPFRs) is very limited in rodents. Our recent publication on exposure of adult mice to two concentrations of PBDE-47 or a mixture of OPFRs (TPP, TCP, TDCPP) at 1 or 10 mg/kg/day for 28 days reported that *Kiss1* expression in the arcuate was increased two-fold in both sexes (intact males and ovariectomized females) (Krumm *et al.* 2018). These transcriptional effects were not observed in ERα knockout mice indicating that these flame retardants may act through estrogen signaling to influence gene expression. However, the change in *Kiss1* expression did not lead to disruption of estrous cyclicity in intact females (Vail *et al.* 2020).

The previous sections discuss the impact of perinatal, juvenile, and adult exposures to EDCs on the HPG axis in rodents. In general, most EDC studies, including those above, use acute or subchronic exposures at concentrations higher than human or wildlife exposures. However, humans and wildlife are not exposed solely during these separate developmental windows but across the entire lifespan, from pre-conception to death. Thus, short-term exposure experiments do not accurately recapitulate the anthropogenic environment. We are not suggesting that these short-term experiments are without value as they are necessary to establish mechanisms and determine the most sensitive windows of exposure. Rather, that continuous exposure studies to lower environmentally relevant doses, although time-consuming and expensive, would more closely reflect real-world exposure and better address the applicability of chronic EDC exposure to fertility issues and human health outcomes.

In vitro exposures

Using immortalized hypothalamic cell lines or hypothalamic explants provide advantages in studying the mechanisms behind the reproductive endocrine disruption. There are only a few studies that have characterized TCDD effects on cells that express GnRH, kisspeptin, and pituitary gonadotropins. In immortalized GnRH cells (GT1-7), TCDD did not alter GnRH content or cell number due to the lack of AhR expression in these cells (Petroff *et al.* 2003). In explants of the hypothalamus and pituitary from adult Sprague–Dawley female rats, TCDD exposure (3.2 nM, 6 h) did not alter baseline GnRH production or GnRH pulsatility nor did TCDD alter baseline gonadotropin production or average peak amplitude (Trewin *et al.* 2007). In cultured pituitary cells from male and female Wistar rats at GD20, incubation with TCDD (1 or 10 nM, 1–24 h) attenuated the GnRH-induced expression of *Lhb* and α*Gsu* potentially via interfering with PKA/PKC signaling (Takeda *et al.* 2011). In rat hypothalamic GnV-3 cells, TCDD (10 nM) exposure dysregulated *Gnrh* expression via period circadian protein homolog 1 (*Per1*) through the actions of AhR (Solak *et al.* 2013). Finally, in human embryonic kidney cells (GripTite™ 293 MSR) transfected with the *Kiss1* promoter constructs and in rat hypothalamic primary cell culture, TCDD (10 mM) suppressed *Kiss1* and *Gnrh* through xenobiotic response element activation (Mueller & Heger 2014). These data indicate that TCDD suppresses both the neuropeptides (GnRH, kisspeptin) and gonadotropins that control the reproductive axis through the activity of AhR and its downstream effectors.

There are few studies examining the effects of PCBs, DDT, or MXC on the hypothalamic-pituitary circuit in cell models. In a study examining GnRH-stimulated gonadotropin production from primary pituitary cultures from Sprague–Dawley female rats, Aroclor 1242 (0.1, 1, 10, and 100 ppm) alone did not increase LH or FSH after 24 h preincubation except at the 100-ppm dose (Jansen *et al.* 1993). However, GnRH-stimulated LH and FSH secretion was augmented after 24 and 48 h incubation with A1242 (10 ppm). A1242 (50 ppm) decreased total LH in the presence of GnRH and total FSH only in the absence of GnRH. A1242 (10 ppm) also increased GnRH-stimulated LH release without E2-coincubation. Finally, in a recent study using mouse pituitary gonadotrope cells (LβT2 cells), p,p′-DDT and MXC stimulated mRNA expression of *Fsh*β and *Lh*β in a dose-dependent manner, and both EDCs at 10^{-7} M induced FSH and LH protein production (Zhou *et al.* 2014). Collectively, these cell culture studies illustrate how estrogenic EDCs can alter genes of the HPG axis leading to disruption of puberty, estrous cyclicity, and reproduction, albeit at concentrations higher than typical human exposures.

Conclusions

Collectively, these studies indicate that EDC exposures impact the expression of GnRH and kisspeptin, influence the pulsatile release of GnRH, and subsequently alter gonadotropin production from the pituitary. These influences can occur from single exposures during

perinatal, juvenile growth, and adulthood at low concentrations and lead to disruption of steroidogenesis, estrous cyclicity, and the onset of puberty (Bourguignon *et al.* 2013). Presumably, these EDC impacts are due to activation or influence of ERs, ARs, or in the case of dioxins and PCBs, AhRs, during the sensitive developmental (late gestation), prepubertal, and young adult periods. Future studies should characterize the role of these individual receptors and target neurons upstream of GnRH neurons such as AVPV kisspeptin and arcuate KNDy neurons. Another potential hypothalamic neuron involved in the control of GnRH is RFamide-related peptide-3 (RFRP-3) neurons, also known as gonadotropin-inhibiting hormone (GnIH) neurons, which act as a brake on both AVPV kisspeptin and GnRH neurons (Acevedo-Rodriguez *et al.* 2018). These neurons are also understudied in regards to exposure to endocrine disruptors. Certainly, further investigation is warranted in rodent models using environmentally relevant doses and exposure models.

More research is needed in rodent models and cell lines at relevant concentrations and throughout the lifespan with greater attention to the mechanism and the neural control of the HPG axis (Fig. 2 summarizes the effects of each class of EDCs on the various hypothalamic region and pituitary). Therefore, we are advocating for investigators to look beyond gene and protein expression in the hypothalamus and the pituitary. Researchers should incorporate classic neural techniques like whole-cell patch-clamp electrophysiology to assess the influence of EDC on GnRH, kisspeptin, and RFRP-3 neuronal activity along with assessments of the innervation of kisspeptin and RFRP-3 inputs on GnRH neurons. We have recently characterized the influence of adult exposures to OPFRs on arcuate melanocortin neurons and found that these neurons are excited by OPFR exposure in female mice (Vail & Roepke 2020), which suggests that other steroid-sensitive hypothalamic neurons may also be a target for adult exposure to EDCs. Another avenue of investigation is assessing LH pulsatility in WT and transgenic mouse models as LH pulsatility can be reliably measured using an ultra-high sensitive LH assay from small amounts (~6 µL) of blood (Steyn *et al.* 2011). These novel studies, when employed together, would assess the influence of EDCs at the level of the synapse and the circuit and the downstream control of LH, all of which are important for assessing the mechanisms of EDC toxicity and its influence on reproduction across species.

Our review has several limitations in regard to relevancy to human health. First, as we have highlighted throughout the review, many of these studies are using concentrations of EDCs higher than what is reported in the home and in occupational or natural environments. Thus, the direct translational relevance is not strong, in that regard. However, due to the continuous nature of human exposures to low concentrations for a wide range of EDCs and for an expanding mixture of chemicals, such short-term or acute studies are necessary to demonstrate potential molecular mechanisms – hormone receptors, steroidogenic enzymes, epigenetic modifications of reproductive genes in the HPG axis, etc. Furthermore, few studies examine the effects of a mixture of EDCs from many classes of chemicals on the HPG axis. This is, in part, due to the limitations made by review panels and funding sources and their focus on the mechanisms instead of the necessary descriptive studies on the influences of multiple EDC exposures. Secondly, we focused on rats and mice as the majority of studies in this field are focused on mammalian reproduction. However, there is an increasing number of studies that use domesticated mammals, fish, and invertebrate animal models (Bellingham *et al.* 2016, Cuvillier-Hot & Lenoir 2020, You & Song 2021), which may be key in determining receptor-mediated mechanisms. As fish are more sensitive to these compounds than rodents, the impact of these EDCs on fish reproduction is a major cause of concern for aquatic species, including commercially relevant fish stocks. These findings can then be compared and integrated into studies in rodents and in epidemiological studies in humans. Finally, the response to EDCs is influenced by multiple factors – sex, age, and diet. Most of these studies and EDC studies focused on young (<6 months) animals, regardless of exposure window, and did not include interactions with diets. Maternal obesity or high-fat diet feeding can disrupt reproduction (Yao *et al.* 2021), and diet-induced obesity in adults also disrupts reproduction (Negrón & Radovick 2020). The interactions of EDC exposures and obesity on reproduction are an understudied area of reproductive toxicology. In summary, these data in rodents and cell

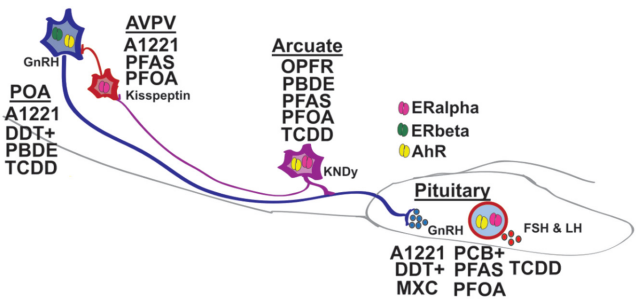

Figure 2 Summary of tissue-specific actions of EDCs on reproductive hormones and neuropeptides. GnRH neurons in the POA are targeted by PCBs, specifically A1221, DDT (and its metabolites), PBDEs, and TCDD. POA GnRH neurons express AhR and Erβ (ERbeta). Kisspeptin neurons in the AVPV are targeted by PCBs, specifically A1221, and PFAS/PFOA and express ERα (ERalpha). KNDy neurons in the arcuate nucleus are targeted by OPFRs, PBDEs, PFAS/PFOA, and TCDD and express AhR and ERα (ERalpha). Pituitary gonadotropes are targeted by A1221, DDT (and its metabolites), MXC, PCBs, PFAS/PFOA, and TCDD. Gonadotropes express AhR and ERα (ERalpha). In some studies, the effects of the EDCs on LH or FSH are due to disruption of steroidogenesis in the gonads and reflect a secondary effect of changes to negative feedback.

lines are highly suggestive that chronic exposures to EDCs in humans may have significant implications for public health and regulatory policy across the globe. Chronic exposures to EDCs across generations will influence the fertility of future generations and lead to an increase in reproductive diseases.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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Author contribution statement

T A R chose the review topic; N C S performed the literature review; T A R and N C S wrote the manuscript.

References

Abbasi G, Buser AM, Soehl A, Murray MW & Diamond ML 2015 Stocks and flows of PBDEs in products from use to waste in the U.S. and Canada from 1970 to 2020. *Environmental Science and Technology* **49** 1521–1528. (https://doi.org/10.1021/es504007v)

Acevedo-Rodriguez A, Kauffman AS, Cherrington BD, Borges CS, Roepke TA & Laconi M 2018 Emerging insights into hypothalamic-pituitary-gonadal (HPG) axis regulation and interaction with stress signaling. *Journal of Neuroendocrinology* **30** e12590. (https://doi.org/10.1111/jne.12590)

Adams S, Wiersielis K, Yasrebi A, Conde K, Armstrong L, Guo GL & Roepke TA 2020 Sex- and age-dependent effects of maternal organophosphate flame-retardant exposure on neonatal hypothalamic and hepatic gene expression. *Reproductive Toxicology* **94** 65–74. (https://doi.org/10.1016/j.reprotox.2020.04.001)

Adgent MA, Hoffman K, Goldman BD, Sjödin A & Daniels JL 2014 Brominated flame retardants in breast milk and behavioural and cognitive development at 36 months. *Paediatric and Perinatal Epidemiology* **28** 48–57. (https://doi.org/10.1111/ppe.12078)

Akinola LK, Uzairu A, Shallangwa GA & Abechi SE 2021 Theoretical study on endocrine disrupting effects of polychlorinated dibenzo-p-dioxins using molecular docking simulation. *Journal of Applied Toxicology* **41** 233–246. (https://doi.org/10.1002/jat.4039)

Austin ME, Kasturi BS, Barber M, Kannan K, Mohankumar PS & Mohankumar SMJ 2003 Neuroendocrine effects of perfluorooctane sulfonate in rats. *Environmental Health Perspectives* **111** 1485–1489. (https://doi.org/10.1289/ehp.6128)

Ballesteros V, Costa O, Iñiguez C, Fletcher T, Ballester F & Lopez-Espinosa MJ 2017 Exposure to perfluoroalkyl substances and thyroid function in pregnant women and children: a systematic review of epidemiologic studies. *Environment International* **99** 15–28. (https://doi.org/10.1016/j.envint.2016.10.015)

Bellingham M, Fowler PA, MacDonald ES, Mandon-Pepin B, Cotinot C, Rhind S, Sharpe RM & Evans NP 2016 Timing of maternal exposure and foetal sex determine the effects of low-level chemical mixture exposure on the foetal neuroendocrine system in sheep. *Journal of Neuroendocrinology* **28**. (https://doi.org/10.1111/jne.12444)

Ben Rhouma K, Tebourbi O, Krichah R & Sakly M 2001 Reproductive toxicity of DDT in adult male rats. *Human and Experimental Toxicology* **20** 393–397. (https://doi.org/10.1191/096032701682692946)

Blum A, Behl M, Birnbaum LS, Diamond ML, Phillips A, Singla V, Sipes NS, Stapleton HM & Venier M 2019 Organophosphate ester flame retardants: are they a regrettable substitution for polybrominated diphenyl ethers? *Environmental Science and Technology Letters* **6** 638–649. (https://doi.org/10.1021/acs.estlett.9b00582)

Bookstaff RC, Kamel F, Moore RW, Bjerke DL & Peterson RE 1990 Altered regulation of pituitary gonadotropin-releasing hormone (GnRH) receptor number and pituitary responsiveness to GnRH in 2,3,7,8-tetrachlorodibenzo-p-dioxin-treated male rats. *Toxicology and Applied Pharmacology* **105** 78–92. (https://doi.org/10.1016/0041-008x(90)90360-7)

Bourguignon JP, Franssen D, Gérard A, Janssen S, Pinson A, Naveau E, Parent AS 2013 Early neuroendocrine disruption in hypothalamus and hippocampus: developmental effects including female sexual maturation and implications for endocrine disrupting chemical screening. *Journal of Neuroendocrinology* **25** 1079–1087. (https://doi.org/10.1111/jne.12107)

Boyle M, Buckley JP & Quirós-Alcalá L 2019 Associations between urinary organophosphate ester metabolites and measures of adiposity among U.S. children and adults: NHANES 2013–2014. *Environment International* **127** 754–763. (https://doi.org/10.1016/j.envint.2019.03.055)

Butt CM, Congleton J, Hoffman K, Fang M & Stapleton HM 2014 Metabolites of organophosphate flame retardants and 2-ethylhexyl tetrabromobenzoate in urine from paired mothers and toddlers. *Environmental Science and Technology* **48** 10432–10438. (https://doi.org/10.1021/es5025299)

Cao J, Patisaul HB & Petersen SL 2011 Aryl hydrocarbon receptor activation in lactotropes and gonadotropes interferes with estradiol-dependent and -independent preprolactin, glycoprotein alpha and luteinizing hormone beta gene expression. *Molecular and Cellular Endocrinology* **333** 151–159. (https://doi.org/10.1016/j.mce.2010.12.027)

Carignan CC, McClean MD, Cooper EM, Watkins DJ, Fraser AJ, Heiger-Bernays W, Stapleton HM & Webster TF 2013 Predictors of tris(1,3-dichloro-2-propyl) phosphate metabolite in the urine of office workers. *Environment International* **55** 56–61. (https://doi.org/10.1016/j.envint.2013.02.004)

Castorina R, Bradman A, Stapleton HM, Butt C, Avery D, Harley KG, Gunier RB, Holland N & Eskenazi B 2017 Current-use flame retardants: maternal exposure and neurodevelopment in children of the CHAMACOS cohort. *Chemosphere* **189** 574–580. (https://doi.org/10.1016/j.chemosphere.2017.09.037)

Clements RJ, Lawrence RC & Blank JL 2009 Effects of intrauterine 2,3,7,8-tetrachlorodibenzo-p-dioxin on the development and function of the gonadotrophin releasing hormone neuronal system in the male rat. *Reproductive Toxicology* **28** 38–45. (https://doi.org/10.1016/j.reprotox.2009.02.002)

Consonni D, Sindaco R & Bertazzi PA 2012 Blood levels of dioxins, furans, dioxin-like PCbs, and TEQs in general populations: a review, 1989–2010. *Environment International* **44** 151–162. (https://doi.org/10.1016/j.envint.2012.01.004)

Coperchini F, Awwad O, Rotondi M, Santini F, Imbriani M & Chiovato L 2017 Thyroid disruption by perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA). *Journal of Endocrinological Investigation* **40** 105–121. (https://doi.org/10.1007/s40618-016-0572-z)

Costa LG & Giordano G 2007 Developmental neurotoxicity of polybrominated diphenyl ether (PBDE) flame retardants. *NeuroToxicology* **28** 1047–1067. (https://doi.org/10.1016/j.neuro.2007.08.007)

Cummings AM 1997 Methoxychlor as a model for environmental estrogens. *Critical Reviews in Toxicology* **27** 367–379. (https://doi.org/10.3109/10408449709089899)

Cuvillier-Hot V & Lenoir A 2020 Invertebrates facing environmental contamination by endocrine disruptors: novel evidences and recent insights. *Molecular and Cellular Endocrinology* **504** 110712. (https://doi.org/10.1016/j.mce.2020.110712)

Desaulniers D, Leingartner K, Wade M, Fintelman E, Yagminas A & Foster WG 1999 Effects of acute exposure to PCbs 126 and 153 on anterior pituitary and thyroid hormones and FSH isoforms in adult Sprague Dawley male rats. *Toxicological Sciences* **47** 158–169. (https://doi.org/10.1093/toxsci/47.2.158)

Dickerson SM & Gore AC 2007 Estrogenic environmental endocrine-disrupting chemical effects on reproductive neuroendocrine function and dysfunction across the life cycle. *Reviews in Endocrine and Metabolic Disorders* **8** 143–159. (https://doi.org/10.1007/s11154-007-9048-y)

Dickerson SM, Cunningham SL & Gore AC 2011*a* Prenatal PCbs disrupt early neuroendocrine development of the rat hypothalamus. *Toxicology and Applied Pharmacology* **252** 36–46. (https://doi.org/10.1016/j.taap.2011.01.012)

Dickerson SM, Cunningham SL, Patisaul HB, Woller MJ & Gore AC 2011*b* Endocrine disruption of brain sexual differentiation by developmental

PCB exposure. *Endocrinology* **152** 581–594. (https://doi.org/10.1210/en.2010-1103)

Dishaw LV, Macaulay LJ, Roberts SC & Stapleton HM 2014 Exposures, mechanisms, and impacts of endocrine-active flame retardants. *Current Opinion in Pharmacology* **19** 125–133. (https://doi.org/10.1016/j.coph.2014.09.018)

Doherty BT, Hoffman K, Keil AP, Engel SM, Stapleton HM, Goldman BD, Olshan AF & Daniels JL 2019 Prenatal exposure to organophosphate esters and behavioral development in young children in the pregnancy, infection, and nutrition study. *NeuroToxicology* **73** 150–160. (https://doi.org/10.1016/j.neuro.2019.03.007)

Domingo JL & Nadal M 2017 Per- and polyfluoroalkyl substances (PFASs) in food and human dietary intake: a review of the recent scientific literature. *Journal of Agricultural and Food Chemistry* **65** 533–543. (https://doi.org/10.1021/acs.jafc.6b04683)

Du G, Hu J, Huang Z, Yu M, Lu C, Wang X & Wu D 2019 Neonatal and juvenile exposure to perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS): advance puberty onset and kisspeptin system disturbance in female rats. *Ecotoxicology and Environmental Safety* **167** 412–421. (https://doi.org/10.1016/j.ecoenv.2018.10.025)

Eskenazi B, Rauch SA, Tenerelli R, Huen K, Holland NT, Lustig RH, Kogut K, Bradman A, Sjödin A & Harley KG 2017 In utero and childhood DDT, DDE, PBDE and PCBs exposure and sex hormones in adolescent boys: the CHAMACOS study. *International Journal of Hygiene and Environmental Health* **220** 364–372. (https://doi.org/10.1016/j.ijheh.2016.11.001)

Faass O, Ceccatelli R, Schlumpf M & Lichtensteiger W 2013 Developmental effects of perinatal exposure to PBDE and PCB on gene expression in sexually dimorphic rat brain regions and female sexual behavior. *General and Comparative Endocrinology* **188** 232–241. (https://doi.org/10.1016/j.ygcen.2013.04.008)

Fan X, Kubwabo C, Rasmussen PE & Wu F 2014 Simultaneous determination of thirteen organophosphate esters in settled indoor house dust and a comparison between two sampling techniques. *Science of the Total Environment* **491–492** 80–86. (https://doi.org/10.1016/j.scitotenv.2013.12.127)

Feng X, Wang X, Cao X, Xia Y, Zhou R & Chen L 2015 Chronic exposure of female mice to an environmental level of perfluorooctane sulfonate suppresses estrogen synthesis through reduced histone h3k14 acetylation of the StAR promoter leading to deficits in follicular development and ovulation. *Toxicological Sciences* **148** 368–379. (https://doi.org/10.1093/toxsci/kfv197)

Fernandes AR & Falandysz J 2021 Science of the total environment polybrominated dibenzo-p-dioxins and furans (PBDD/Fs): contamination in food, humans and dietary exposure. *Science of the Total Environment* **761** 143191. (https://doi.org/10.1016/j.scitotenv.2020.143191)

Gao X, Petroff BK, Rozman KK & Terranova PF 2000 Gonadotropin-releasing hormone (GnRH) partially reverses the inhibitory effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on ovulation in the immature gonadotropin-treated rat. *Toxicology* **147** 15–22. (https://doi.org/10.1016/s0300-483x(00)00161-x)

García-Galiano D, Pinilla L & Tena-Sempere M 2012 Sex steroids and the control of the Kiss1 system: developmental roles and major regulatory actions. *Journal of Neuroendocrinology* **24** 22–33. (https://doi.org/10.1111/j.1365-2826.2011.02230.x)

Gilpin RK, Wagel DJ & Solch JG 2003 Production, distribution, and fate of polychlorinated dibenzo-p-dioxins, dibenzofurans, and related organohalogens in the environment. In *Dioxins and Health*, pp. 55–87. John Wiley & Sons, Ltd.

Gore AC, Walker DM, Zama AM, Armenti AE & Uzumcu M 2011 Early life exposure to endocrine-disrupting chemicals causes lifelong molecular reprogramming of the hypothalamus and premature reproductive aging. *Molecular Endocrinology* **25** 2157–2168. (https://doi.org/10.1210/me.2011-1210)

Graceli JB, Dettogni RS, Merlo E, Niño O, da Costa CS, Zanol JF, Ríos Morris EA, Miranda-Alves L & Denicol AC 2020 The impact of endocrine-disrupting chemical exposure in the mammalian hypothalamic-pituitary axis. *Molecular and Cellular Endocrinology* **518** 110997. (https://doi.org/10.1016/j.mce.2020.110997)

Gravel S, Lavoué J, Bakhiyi B, Lavoie J, Roberge B, Patry L, Bouchard MF, Verner MA, Zayed J & Labrèche F 2020 Multi-exposures to suspected endocrine disruptors in electronic waste recycling workers: associations with thyroid and reproductive hormones. *International Journal of Hygiene and Environmental Health* **225** 113445. (https://doi.org/10.1016/j.ijheh.2019.113445)

Hamm JT, Sparrow BR, Wolf D & Birnbaum LS 2000 In utero and lactational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin alters postnatal development of seminal vesicle epithelium. *Toxicological Sciences* **54** 424–430. (https://doi.org/10.1093/toxsci/54.2.424)

Han DY, Kang SR, Park OS, Cho JH, Won CK, Park HS, Park KII, Kim EH & Kim GS 2010 Polychlorinated biphenyls have inhibitory effect on testicular steroidogenesis by downregulation of P45017α and P450scc. *Toxicology and Industrial Health* **26** 287–296. (https://doi.org/10.1177/0748233710364961)

Hewitt SC & Korach KS 2011 Estrogenic activity of bisphenol A and 2,2-bis(p-hydroxyphenyl)-1,1,1-trichloroethane (HPTE) demonstrated in mouse uterine gene profiles. *Environmental Health Perspectives* **119** 63–70. (https://doi.org/10.1289/ehp.1002347)

Hoffman K, Daniels JL & Stapleton HM 2014 Urinary metabolites of organophosphate flame retardants and their variability in pregnant women. *Environment International* **63** 169–172. (https://doi.org/10.1016/j.envint.2013.11.013)

Hoffman K, Butt CM, Chen A, Limkakeng AT & Stapleton HM 2015 High exposure to organophosphate flame retardants in infants: associations with baby products. *Environmental Science and Technology* **49** 14554–14559. (https://doi.org/10.1021/acs.est.5b03577)

Hoffman K, Butt CM, Webster TF, Preston EV, Hammel SC, Makey C, Lorenzo AM, Cooper EM, Carignan C, Meeker JD *et al.* 2017 Temporal trends in exposure to organophosphate flame retardants in the United States. *Environmental Science and Technology Letters* **4** 112–118. (https://doi.org/10.1021/acs.estlett.6b00475)

Hoffman K, Stapleton HM, Lorenzo A, Butt CM, Adair L, Herring AH & Daniels JL 2018 Prenatal exposure to organophosphates and associations with birthweight and gestational length. *Environment International* **116** 248–254. (https://doi.org/10.1016/j.envint.2018.04.016)

Hu XN, Zhang JQ, Jiang YS, Lei YX, Lu LG, Zhou J, Huang HY, Fang D & Tao GH 2014 Effect on metabolic enzymes and thyroid receptors induced by BDE-47 by activation the pregnane X receptor in HepG2, a human hepatoma cell line. *Toxicology in Vitro* **28** 1377–1385. (https://doi.org/10.1016/j.tiv.2014.07.004)

Huang L, Huang R, Ran XR, Liu HY, Zhang Y, Dai LJ & Li B 2011 Three-generation experiment showed female C57BL/6J mice drink drainage canal water containing low level of TCDD-like activity causing high pup mortality. *Journal of Toxicological Sciences* **36** 713–724. (https://doi.org/10.2131/jts.36.713)

Huo X, Chen D, He Y, Zhu W, Zhou W & Zhang J 2015 Bisphenol-A and female infertility: a possible role of gene-environment interactions. *International Journal of Environmental Research and Public Health* **12** 11101–11116. (https://doi.org/10.3390/ijerph120911101)

Jansen HT, Cooke PS, Porcelli J, Liu TC & Hansen LG 1993 Estrogenic and antiestrogenic actions of PCbs in the female rat: in vitro and in vivo studies. *Reproductive Toxicology* **7** 237–248. (https://doi.org/10.1016/0890-6238(93)90230-5)

Kakeyama M, Sone H & Tohyama C 2008 Perinatal exposure of female rats to 2,3,7,8-tetrachlorodibenzo-p-dioxin induces central precocious puberty in the offspring. *Journal of Endocrinology* **197** 351–358. (https://doi.org/10.1677/JOE-08-0062)

Kalil B, Ribeiro AB, Leite CM, Uchôa ET, Carolino RO, Cardoso TSR, Elias LLK, Rodrigues JA, Plant TM, Poletini MO *et al.* 2016 The increase in signaling by kisspeptin neurons in the preoptic area and associated changes in clock gene expression that trigger the LH surge in female rats are dependent on the facilitatory action of a noradrenaline input. *Endocrinology* **157** 323–335. (https://doi.org/10.1210/en.2015-1323)

Kim J-WW, Isobe T, Muto M, Tue NM, Katsura K, Malarvannan G, Sudaryanto A, Chang K-HH, Prudente M, Viet PH *et al.* 2014 Organophosphorus flame retardants (PFRs) in human breast milk from several Asian countries. *Chemosphere* **116** 91–97. (https://doi.org/10.1016/j.chemosphere.2014.02.033)

Koh WX, Hornbuckle KC & Thorne PS 2015 Human serum from urban and rural adolescents and their mothers shows exposure to polychlorinated biphenyls not found in commercial mixtures. *Environmental Science and Technology* **49** 8105–8112. (https://doi.org/10.1021/acs.est.5b01854)

Krumm EA, Patel VJ, Tillery TS, Yasrebi A, Shen J, Guo GL, Marco SM, Buckley BT & Roepke TA 2018 Organophosphate flame-retardants alter adult mouse homeostasis and gene expression in a sex-dependent manner potentially through interactions with ERα. *Toxicological Sciences* **162** 212–224. (https://doi.org/10.1093/toxsci/kfx238)

La Merrill MA, Vandenberg LN, Smith MT, Goodson W, Browne P, Patisaul HB, Guyton KZ, Kortenkamp A, Cogliano VJ, Woodruff TJ *et al.* 2020 Consensus on the key characteristics of endocrine-disrupting chemicals as a basis for hazard identification. *Nature Reviews: Endocrinology* **16** 45–57. (https://doi.org/10.1038/s41574-019-0273-8)

Lafuente A, Cabaleiro T, Caride A, Esquifino AI 2008 Toxic effects of methoxychlor administered subcutaneously on the hypothalamic-pituitary-testicular axis in adult rats. *Food and Chemical Toxicology* **46** 1570–1575. (https://doi.org/10.1016/j.fct.2007.12.017)

Larsson M, Van Den Berg M, Brenerová P, Van Duursen MBM, Van Ede KI, Lohr C, Luecke-Johansson S, Machala M, Neser S, Pěnčíková K *et al.* 2015 Consensus toxicity factors for polychlorinated dibenzo-P-dioxins, dibenzofurans, and biphenyls combining in silico models and extensive in vitro screening of AhR-mediated effects in human and rodent cells. *Chemical Research in Toxicology* **28** 641–650. (https://doi.org/10.1021/tx500434j)

Leonetti C, Butt CM, Hoffman K, Hammel SC, Miranda ML & Stapleton HM 2016 Brominated flame retardants in placental tissues: associations with infant sex and thyroid hormone endpoints. *Environmental Health : a Global Access Science Source* **15** 113. (https://doi.org/10.1186/s12940-016-0199-8)

León-Olea M, Martyniuk CJ, Orlando EF, Ottinger MA, Rosenfeld CS, Wolstenholme JT & Trudeau VL 2014 Current concepts in neuroendocrine disruption. *General and Comparative Endocrinology* **203** 158–173. (https://doi.org/10.1016/j.ygcen.2014.02.005)

Li X, Johnson DC & Rozman KK 1997 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) increases release of luteinizing hormone and follicle-stimulating hormone from the pituitary of immature female rats in vivo and in vitro. *Toxicology and Applied Pharmacology* **142** 264–269. (https://doi.org/10.1006/taap.1996.8044)

Liu G, Dhana K, Furtado JD, Rood J, Zong G, Liang L, Qi L, Bray GA, DeJonge L, Coull B *et al.* 2018 Perfluoroalkyl substances and changes in body weight and resting metabolic rate in response to weight-loss diets: a prospective study. *PLoS Medicine* **15** e1002502. (https://doi.org/10.1371/journal.pmed.1002502)

Longnecker MP, Klebanoff MA, Zhou H & Brock JW 2001 Association between maternal serum concentration of the DDT metabolite DDE and preterm and small-for-gestational-age babies at birth. *Lancet* **358** 110–114. (https://doi.org/10.1016/S0140-6736(01)05329-6)

López-Doval S, Salgado R, Pereiro N, Moyano R & Lafuente A 2014 Perfluorooctane sulfonate effects on the reproductive axis in adult male rats. *Environmental Research* **134** 158–168. (https://doi.org/10.1016/j.envres.2014.07.006)

López-Doval S, Salgado R, Fernández-Pérez B & Lafuente A 2015 Possible role of serotonin and neuropeptide Y on the disruption of the reproductive axis activity by perfluorooctane sulfonate. *Toxicology Letters* **233** 138–147. (https://doi.org/10.1016/j.toxlet.2015.01.012)

López-Doval S, Salgado R & Lafuente A 2016 The expression of several reproductive hormone receptors can be modified by perfluorooctane sulfonate (PFOS) in adult male rats. *Chemosphere* **155** 488–497. (https://doi.org/10.1016/j.chemosphere.2016.04.081)

Makita Y 2008 Effects of perinatal combined exposure to 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (p,p′-DDE) and tributyltin (TBT) on rat female reproductive system. *Environmental Toxicology and Pharmacology* **25** 380–385. (https://doi.org/10.1016/j.etap.2007.12.003)

Martyniuk CJ, Mehinto AC & Denslow ND 2020 Organochlorine pesticides: agrochemicals with potent endocrine-disrupting properties in fish. *Molecular and Cellular Endocrinology* **507** 110764. (https://doi.org/10.1016/j.mce.2020.110764)

Masutomi N, Shibutani M, Takagi H, Uneyama C, Lee KY & Hirose M 2004 Alteration of pituitary hormone-immunoreactive cell populations in rat offspring after maternal dietary exposure to endocrine-active chemicals. *Archives of Toxicology* **78** 232–240. (https://doi.org/10.1007/s00204-003-0528-x)

McIntyre JK & Beauchamp DA 2007 Age and trophic position dominate bioaccumulation of mercury and organochlorines in the food web of Lake Washington. *Science of the Total Environment* **372** 571–584. (https://doi.org/10.1016/j.scitotenv.2006.10.035)

Meeker JD, Cooper EM, Stapleton HM & Hauser R 2013 Urinary metabolites of organophosphate flame retardants: temporal variability and correlations with house dust concentrations. *Environmental Health Perspectives* **121** 580–585. (https://doi.org/10.1289/ehp.1205907)

Mueller JK & Heger S 2014 Endocrine disrupting chemicals affect the gonadotropin releasing hormone neuronal network. *Reproductive Toxicology* **44** 73–84. (https://doi.org/10.1016/j.reprotox.2013.10.011)

Mutoh J, Taketoh J, Okamura K, Kagawa T, Ishida T, Ishii Y & Yamada H 2006 Fetal pituitary gonadotropin as an initial target of dioxin in its impairment of cholesterol transportation and steroidogenesis in rats. *Endocrinology* **147** 927–936. (https://doi.org/10.1210/en.2005-1125)

Negrón AL & Radovick S 2020 High-fat diet alters LH secretion and pulse frequency in female mice in an estrous cycle-dependent manner. *Endocrinology* **161** 1–9. (https://doi.org/10.1210/endocr/bqaa146)

Owen BM, Bookout AL, Ding X, Lin VY, Atkin SD, Gautron L, Kliewer SA & Mangelsdorf DJ 2013 FGF21 contributes to neuroendocrine control of female reproduction. *Nature Medicine* **19** 1153–1156. (https://doi.org/10.1038/nm.3250)

Patisaul HB, Fenton SE & Aylor DL 2018 Animal models of endocrine disruption. *Best Practice and Research: Clinical Endocrinology and Metabolism* **32** 283–297. (https://doi.org/10.1016/j.beem.2018.03.011)

Percy Z, La Guardia MJ, Xu Y, Hale RC, Dietrich KN, Lanphear BP, Yolton K, Vuong AM, Cecil KM, Braun JM *et al.* 2020 Concentrations and loadings of organophosphate and replacement brominated flame retardants in house dust from the home study during the PBDE phase-out. *Chemosphere* **239** 124701. (https://doi.org/10.1016/j.chemosphere.2019.124701)

Pessah IN, Cherednichenko G & Lein PJ 2010 Minding the calcium store: ryanodine receptor activation as a convergent mechanism of PCB toxicity. *Pharmacology and Therapeutics* **125** 260–285. (https://doi.org/10.1016/j.pharmthera.2009.10.009)

Petroff BK, Croutch CR, Hunter DM, Wierman ME & Gao X 2003 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) stimulates gonadotropin secretion in the immature female Sprague-Dawley rat through a pentobarbital-and estradiol-sensitive mechanism but does not alter gonadotropin-releasing hormone (GnRH) secretion by immortal. *Biology of Reproduction* **68** 2100–2106. (https://doi.org/10.1095/biolreprod.102.010439)

Pivonello C, Muscogiuri G, Nardone A, Garifalos F, Provvisiero DP, Verde N, Angelis C De, Conforti A, Piscopo M, Auriemma RS *et al.* 2020 Bisphenol A: an emerging threat to female fertility. *Reproductive Biology and Endocrinology* **18** 22. (https://doi.org/10.1186/s12958-019-0558-8)

Rasier G, Parent AS, Gérard A, Lebrethon MC & Bourguignon JP 2007 Early maturation of gonadotropin-releasing hormone secretion and sexual precocity after exposure of infant female rats to estradiol or dichlorodiphenyltrichloroethane. *Biology of Reproduction* **77** 734–742. (https://doi.org/10.1095/biolreprod.106.059303)

Rasier G, Parent AS, Gérard A, Denooz R, Lebrethon MC, Charlier C & Bourguignon JP 2008 Mechanisms of interaction of endocrine-disrupting chemicals with glutamate-evoked secretion of gonadotropin-releasing hormone. *Toxicological Sciences* **102** 33–41. (https://doi.org/10.1093/toxsci/kfm285)

Rodríguez-Alcalá LM, Sá C, Pimentel LL, Pestana D, Teixeira D, Faria A, Calhau C & Gomes A 2015 Endocrine disruptor DDE associated with a high-fat diet enhances the impairment of liver fatty acid composition in rats. *Journal of Agricultural and Food Chemistry* **63** 9341–9348. (https://doi.org/10.1021/acs.jafc.5b03274)

Roepke TA, Yang JA, Yasrebi A, Mamounis KJ, Oruc E, Zama AM & Uzumcu M 2016 Regulation of arcuate genes by developmental exposures to endocrine-disrupting compounds in female rats. *Reproductive Toxicology* **62** 18–26. (https://doi.org/10.1016/j.reprotox.2016.04.014)

Santoro A, Chianese R, Troisi J, Richards S, Nori SL, Fasano S, Guida M, Plunk E, Viggiano A, Pierantoni R *et al.* 2019 Neuro-toxic and reproductive effects of BPA. *Current Neuropharmacology* **17** 1109–1132. (https://doi.org/10.2174/1570159X17666190726112101)

Schecter A, Birnbaum L, Ryan JJ & Constable JD 2006 Dioxins: an overview. *Environmental Research* **101** 419–428. (https://doi.org/10.1016/j.envres.2005.12.003)

Shi Z, Valdez KE, Ting AY, Franczak A, Gum SL & Petroff BK 2007 Ovarian endocrine disruption underlies premature reproductive senescence following environmentally relevant chronic exposure to the aryl hydrocarbon receptor agonist 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Biology of Reproduction* **76** 198–202. (https://doi.org/10.1095/biolreprod.106.053991)

Sinchak K, Mohr MA & Micevych PE 2020 Hypothalamic astrocyte development and physiology for neuroprogesterone induction of the luteinizing hormone surge. *Frontiers in Endocrinology* **11** 420. (https://doi.org/10.3389/fendo.2020.00420)

Solak KA, Wijnolts FMJ, Pralong FP, Blaauboer BJ, van den Berg M, Westerink RH & van Duursen MBM 2013 In vitro neuroendocrine effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the AhR-expressing hypothalamic rat GnV-3 cell line. *Toxicology* **311** 124–134. (https://doi.org/10.1016/j.tox.2013.07.006)

Steinberg RM, Walker DM, Juenger TE, Woller MJ & Gore AC 2008 Effects of perinatal polychlorinated biphenyls on adult female rat reproduction: development, reproductive physiology, and second generational effects. *Biology of Reproduction* **78** 1091–1101. (https://doi.org/10.1095/biolreprod.107.067249)

Steyn FJ, Huang L, Ngo ST, Leong JW, Tan HY, Xie TY, Parlow AF, Veldhuis JD, Waters MJ & Chen C 2011 Development of a method for the determination of pulsatile growth hormone secretion in mice. *Endocrinology* **152** 3165–3171. (https://doi.org/10.1210/en.2011-0253)

Sundkvist AM, Olofsson U & Haglund P 2010 Organophosphorus flame retardants and plasticizers in marine and fresh water biota and in human milk. *Journal of Environmental Monitoring* **12** 943–951. (https://doi.org/10.1039/b921910b)

Suzuki M, Lee HC, Chiba S, Yonezawa T & Nishihara M 2004 Effects of methoxychlor exposure during perinatal period on reproductive function after maturation in rats. *Journal of Reproduction and Development* **50** 455–461. (https://doi.org/10.1262/jrd.50.455)

Takeda T, Yamamoto M, Himeno M, Takechi S, Yamaguchi T, Ishida T, Ishii Y & Yamada H 2011 2,3,7,8-Tetrachlorodibenzo-p-dioxin potentially attenuates the gene expression of pituitary gonadotropin β-subunits in a fetal age-specific fashion: a comparative study using cultured pituitaries. *Journal of Toxicological Sciences* **36** 221–229. (https://doi.org/10.2131/jts.36.221)

Takeda T, Fujii M, Hattori Y, Yamamoto M, Shimazoe T, Ishii Y, Himeno M & Yamada H 2014 Maternal exposure to dioxin imprints sexual immaturity of the pups through fixing the status of the reduced expression of hypothalamic gonadotropin- releasing hormone. *Molecular Pharmacology* **85** 74–82. (https://doi.org/10.1124/mol.113.088575)

Tena-Sempere M 2010 Kisspeptin/GPR54 system as potential target for endocrine disruption of reproductive development and function. *International Journal of Andrology* **33** 360–368. (https://doi.org/10.1111/j.1365-2605.2009.01012.x)

Terasawa E 2019 Mechanism of pulsatile GnRH release in primates: unresolved questions. *Molecular and Cellular Endocrinology* **498** 110578. (https://doi.org/10.1016/j.mce.2019.110578)

Tiemann U 2008 In vivo and in vitro effects of the organochlorine pesticides DDT, TCPM, methoxychlor, and lindane on the female reproductive tract of mammals: a review. *Reproductive Toxicology* **25** 316–326. (https://doi.org/10.1016/j.reprotox.2008.03.002)

Tomic D, Frech MS, Babus JK, Gupta RK, Furth PA, Koos RD & Flaws JA 2006 Methoxychlor induces atresia of antral follicles in ERα-overexpressing mice. *Toxicological Sciences* **93** 196–204. (https://doi.org/10.1093/toxsci/kfl040)

Trewin AL, Woller MJ, Wimpee BAB, Conley LK, Baldridge MG & Hutz RJ 2007 Short-term hormone release from adult female rat hypothalamic and pituitary explants is not altered by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Journal of Reproduction and Development* **53** 765–775. (https://doi.org/10.1262/jrd.18101)

Turusov V, Rakitsky V & Tomatis L 2002 Dichlorodiphenyltrichloroethane (DDT): ubiquity, persistence, and risks. *Environmental Health Perspectives* **110** 125–128. (https://doi.org/10.1289/ehp.02110125)

Uslu U, Sandal S, Cumbul A, Yildiz S, Aydin M & Yilmaz B 2013 Evaluation of estrogenic effects of polychlorinated biphenyls and organochlorinated pesticides using immature rat uterotrophic assay. *Human and Experimental Toxicology* **32** 476–482. (https://doi.org/10.1177/0960327112472999)

Vail GM & Roepke TA 2020 Organophosphate flame retardants excite arcuate melanocortin circuitry and increase neuronal sensitivity to ghrelin in adult mice. *Endocrinology* **161** 1–19. (https://doi.org/10.1210/endocr/bqaa168)

Vail GM, Walley SN, Yasrebi A, Maeng A, Conde KM & Roepke TA 2020 The interactions of diet-induced obesity and organophosphate flame retardant exposure on energy homeostasis in adult male and female mice. *Journal of Toxicology and Environmental Health: Part A* **83** 438–455. (https://doi.org/10.1080/15287394.2020.1777235)

van der Veen I & de Boer J 2012 Phosphorus flame retardants: properties, production, environmental occurrence, toxicity and analysis. *Chemosphere* **88** 1119–1153. (https://doi.org/10.1016/j.chemosphere.2012.03.067)

Wang X, Bai Y, Tang C, Cao X, Chang F & Chen L 2018 Impact of perfluorooctane sulfonate on reproductive ability of female mice through suppression of estrogen receptor α-activated kisspeptin neurons. *Toxicological Sciences* **165** 475–486. (https://doi.org/10.1093/toxsci/kfy167)

Waye A & Trudeau VL 2011 Neuroendocrine disruption: more than hormones are upset. *Journal of Toxicology and Environmental Health: Part B, Critical Reviews* **14** 270–291. (https://doi.org/10.1080/10937404.2011.578273)

White SS & Birnbaum LS 2009 An overview of the effects of dioxins and dioxin-like compounds on vertebrates, as documented in human and ecological epidemiology. *Journal of Environmental Science and Health: Part C, Environmental Carcinogenesis and Ecotoxicology Reviews* **27** 197–211. (https://doi.org/10.1080/10590500903310047)

Wong KH & Durrani TS 2017 Exposures to endocrine disrupting chemicals in consumer products – a guide for pediatricians. *Current Problems in Pediatric and Adolescent Health Care* **47** 107–118. (https://doi.org/10.1016/j.cppeds.2017.04.002)

Wu X, Chen A, Yuan Z, Kang H & Xie Z 2020 Atmospheric organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) in the Antarctic marginal seas: distribution, sources and transportation. *Chemosphere* **258** 127359. (https://doi.org/10.1016/j.chemosphere.2020.127359)

Xu JP, Yin YP & Zhou XQ 2008 Effect of vitamin E on reproductive function in the mice treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Toxicology and Industrial Health* **24** 595–601. (https://doi.org/10.1177/0748233708100092)

Xue J, Zartarian V, Moya J, Freeman N, Beamer P, Black K, Tulve N & Shalat S 2007 A meta-analysis of children's hand-to-mouth frequency data for estimating nondietary ingestion exposure. *Risk Analysis* **27** 411–420. (https://doi.org/10.1111/j.1539-6924.2007.00893.x)

Yang J, Zhao Y, Li M, Du M, Li X & Li Y 2019 A review of a class of emerging contaminants: the classification, distribution, intensity of consumption, synthesis routes, environmental effects and expectation of pollution abatement to organophosphate flame retardants (OPFRs). *International Journal of Molecular Sciences* **20** 2874–2912. (https://doi.org/10.3390/ijms20122874)

Yao S, Lopez-Tello J & Sferruzzi-Perri AN 2021 Developmental programming of the female reproductive system – a review. *Biology of Reproduction* **104** 745–770. (https://doi.org/10.1093/biolre/ioaa232)

Yin HP, Xu JP, Zhou XQ & Wang Y 2012 Effects of vitamin E on reproductive hormones and testis structure in chronic dioxin-treated mice. *Toxicology and Industrial Health* **28** 152–161. (https://doi.org/10.1177/0748233711408381)

You HH & Song G 2021 Review of endocrine disruptors on male and female reproductive systems. *Comparative Biochemistry and Physiology: Toxicology and Pharmacology* **244** 109002. (https://doi.org/10.1016/j.cbpc.2021.109002)

Zhang Y, Cao X, Chen L, Qin Y, Xu Y, Tian Y & Chen L 2020 Exposure of female mice to perfluorooctanoic acid suppresses hypothalamic kisspeptin-reproductive endocrine system through enhanced hepatic fibroblast growth factor 21 synthesis, leading to ovulation failure and prolonged dioestrus. *Journal of Neuroendocrinology* **32** e12848. (https://doi.org/10.1111/jne.12848)

Zhao Y, Tan YS, Strynar MJ, Perez G, Haslam SZ & Yang C 2012 Perfluorooctanoic acid effects on ovaries mediate its inhibition of peripubertal mammary gland development in Balb/c and C57BL/6 mice. *Reproductive Toxicology* **33** 563–576. (https://doi.org/10.1016/j.reprotox.2012.02.004)

Zhou J, Yang Y, Xiong K & Liu J 2014 Endocrine disrupting effects of dichlorodiphenyltrichloroethane analogues on gonadotropin hormones in pituitary gonadotrope cells. *Environmental Toxicology and Pharmacology* **37** 1194–1201. (https://doi.org/10.1016/j.etap.2014.04.018)

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